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Invited Review

Root phenotyping: important and minimum information required for root modeling in crop plants

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As plants cannot relocate, they require effective root systems for water and nutrient uptake. Root development plasticity enables plants to adapt to different environmental conditions. Research on improvements in crop root systems is limited in comparison with that in shoots as the former are difficult to image. Breeding more effective root systems is proposed as the "second green revolution". There are several recent publications on root system architecture (RSA), but the methods used to analyze the RSA have not been standardized. Here, we introduce traditional and current root-imaging methods and discuss root structure phenotyping. Some important root structures have not been standardized as roots are easily affected by rhizosphere conditions and exhibit greater plasticity than shoots; moreover, root morphology significantly varies even in the same genotype. For these reasons, it is difficult to define the ideal root systems for breeding. In this review, we introduce several types of software to analyze roots and identify important root parameters by modeling to simplify the root system characterization. These parameters can be extracted from photographs captured in the field. This modeling approach is applicable to various legacy root data stored in old or unpublished formats. Standardization of RSA data could help estimate root ideotypes.

Key Words: legacy data, root imaging, root modeling, root phenotyping, root system architecture.

Introduction

Plant structure considerably varies under different growing conditions even when the plants have the same genotype. Plant architecture is strongly affected by environmental factors such as temperature and light intensity (Tardieu *et al.* 2017). Hence, plant phenomic studies are more difficult than animal phenomic studies. To increase crop yield, shoot traits such as plant height, flowering time, and tiller and flower number have been improved through breeding (Horton 2000, Mathan *et al.* 2016). In contrast, plant roots have received relatively limited attention despite the importance of the root system for water and nutrient uptake by plants. The first green revolution was based on growing crops in soil and applying chemical fertilizer at high rates (Tilman 1998). The second green revolution could be based on crop tolerance to low soil fertility because drought and low soil fertility are the primary causes of low crop yield in developing countries (Lynch 2007). As plant roots widely penetrate the soil and are vital for nutrient acquisition, improvements in root architecture could contribute to the second green revolution. Root system architecture (RSA) consists of the shape and spatial arrangement of root systems within the soil. It is determined by plant genetics and soil environment characteristics such as water and nutrient availability and rhizosphere size (Rogers and Benfey 2015).

Elucidating the RSA could help to understand how plants adapt under changing environments and to improve agricultural productivity. However, there are challenges in evaluating plant root systems. First, the root system cannot be assessed without destroying or losing at least a part of it. Second, the ideal root systems for optimal crop growth are unknown as they vary with environmental conditions. Several approaches have been used for phenotyping root systems and various methods including both nondestructive and effective methods have been reported (Atkinson *et al.* 2019, Guimarães *et al.* 2020, McGrail *et al.* 2020, Tardieu *et al.* 2017).

In this review, we introduce conventional and current methods used for root phenotyping. Data on RSA have accumulated owing to the progress in root phenotyping and analytical software development. However, RSA comprises several root traits that are easily influenced by the changes in environmental conditions. Here, we discuss the minimum

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root trait information required to explain the RSA effectively. To this end, we reconstructed the RSA by root modeling. Recently, root phenotyping pipelines were established (Atkinson *et al.* 2019, Paez-Garcia *et al.* 2015, Tardieu *et al.* 2017) and several root models were constructed (Barczi *et al.* 2018, Postma *et al.* 2017, Schnepf *et al.* 2018). However, scientists may nonetheless retain legacy root data in old or unpublished formats. The minimum information presented in this review could render such data accessible and clarify personal researcher data.

Root system architecture (RSA) phenotyping

Root phenotyping pipelines consist of root imaging and root trait digitization. In this review, we focus exclusively on root system visualization. Several root imaging methods have been reported but there is no standard methodology as each method has its advantages and disadvantages (McGrail *et al.* 2020). Before starting RSA studies, it is necessary to establish whether the plants are grown under field or controlled conditions. Under field conditions, shoot phenotyping in high-throughput platforms and in field-based experiments has improved via advanced remote sensing technologies involving drones and sensors (Chen *et al.* 2019, Liu *et al.* 2019, Tardieu *et al.* 2017). In high-throughput phenotyping, root assessments are less advanced than shoot assessments as it is difficult to visualize underground field areas. Weaver *et al.* (1922) used trenches to expose the RSA under field conditions. The root images were detailed but some trenches were ≥2 m deep and the roots had to be manually excavated. Since then, trenching methods have improved, but root image acquisition is nonetheless time-consuming and trenches are unsuitable for large-scale experiments. To minimize the time for root measurements, a convolutional neural network was applied for the root segmentation in trench profile images (Teramoto and Uga 2020). The predictions of roots using a trained model showed a high correlation with the manually traced results. The application of a deep neural network to root segmentation will improve root image acquisition in other fields experiments (Teramoto and Uga 2020). Soil core sampling is another classical root phenotyping method. Soil cores of length 1–2 m are collected using cylinders several centimeters in diameter. The soil cores are then rinsed, the roots are collected and their distribution is estimated (Kücke *et al.* 1995). Soil cores have also been subjected to fluorescence imaging to obtain high-contrast root images and improve throughput for root segment visualization and measurements (Wasson *et al.* 2016). However, soil cores furnish limited root system data as they provide only partial root images and cannot estimate the whole root system. Moreover, as the roots are segmented when they are collected, it is difficult to distinguish between the roots of target plants and those of the neighboring plants. Monolith methods are also traditionally used for root samplings. The monolith methods involve driving large boxes or cylinders with bottom side open into the ground (Teramoto *et al.* 2020, Wu and Guo 2014). The insertion of monoliths into the soils is difficult because of the hardness of soil, sometimes necessitating the use of hammer or backhoe (Teramoto *et al.* 2020, Wu and Guo 2014). The size of a monolith is larger than that of a cylinder in soil core sampling; a cylinder of diameter 50 cm was used for maize root sampling (Wu and Guo 2014). Minirhizotrons have been used in nondestructive root phenotyping in the field. Plexiglas, cellulose acetate butyrate (CAB), polycarbonate (lexan), acrylic, and glass minirhizotron tubes are inserted into the soil before planting. Color micro-video cameras are inserted into the tubes to capture images of the roots in direct contact with the tubes (Johnson *et al.* 2001). The images provide information about the roots and surrounding environment including the soil and microorganisms. Nevertheless, the captured images only show parts of the root systems, and they do not reflect them in their entirety. Another potential nondestructive root phenotyping method is ground-penetrating radar. However, considering the resolution, its application would be limited to trees and other woody plants (*Populus* × *canadensis*, *Pinus pinaster* Ait. and *P. pinea* L.; Zenone *et al.* 2008).

Shovelomics has been used to phenotype maize crown and brace roots (Trachsel *et al.* 2011). Root number, angle, and branching pattern have been assessed using recombinant inbred lines (RILs; Trachsel *et al.* 2011). However, there is no clear definition for shovelomics and it is uncertain whether it can be applied to other crops besides maize. Here, we define shovelomics as a method of simply digging out soil with a shovel and performing high-throughput RSA phenotyping in the field. Shovelomics could be applied to large-scale experiments, and it is widely use in the RSA analysis of several crops (e.g., rapeseed/canola, common bean, and cowpea). However, it only allows partial RSA assessment as deep roots and thin, soft lateral roots are easily missed during digging. One possible strategy for minimizing these losses and obtaining clear RSA images is to consider the type of soil used for cultivation. For this review, we used a field in the Arid Land Research Center of Tottori University (**Fig. 1A**, **1B**). The soil therein is adjacent to the Tottori sand dune and consists almost entirely of sand with only small proportions of silt and clay (Kimura *et al.* 2004). Sandy soils with a low clay content have a low water holding capacity, and their water content declines more rapidly than that of high clay soils (Yu *et al.* 2017). Although sand dune soil water content quickly decreases, irrigation readily restores and maintains soil moisture. Moreover, it is easy to excavate roots from this type of soil (**Fig. 1C**). It is also easy to rinse arenaceous soil from roots and minimize root loss (**Fig. 1C**, **1D**). The RSA of soybean (*Glycine max*) grown in a sand dune field did not match that of soybean grown hydroponically (**Fig. 1E**). Plant growth is easily influenced by short-term environmental stimuli that may alter morphology, organ and tissue structure, or both. Microscale soil water content patterns (Bao

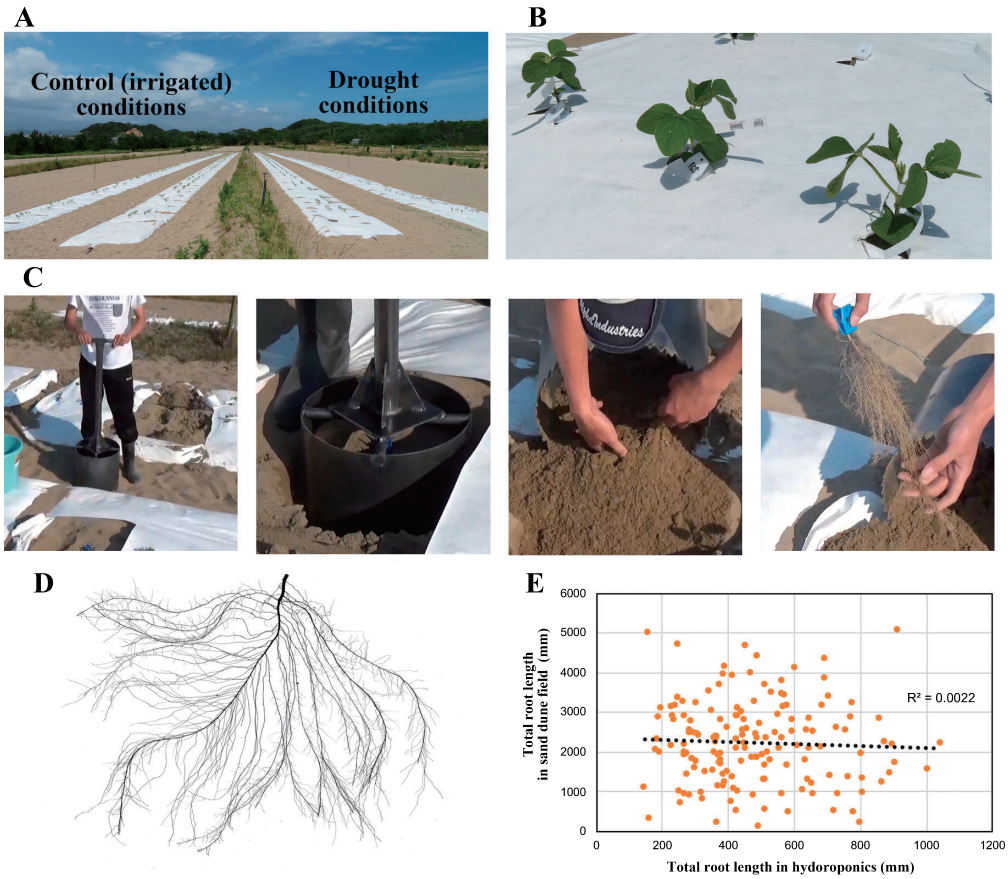

Fig. 1. Large-scale sand dune field experiment. The experimental field consisted mainly of sand, and therefore, its water content was easy to control. (A) Separation of field into irrigated (control) and drought conditions. (B) Soybean (*Glycine max*) seedlings grown in sand dune field. (C) Root sampling in the field of ALRC. Digging tool like a cylinder-monolith was inserted into the soil, and then the root system was collected. The tool can be easily inserted into the sand dune fields. Root loss was minimized because the sand was easily removed from the roots. Only a few minutes are required for root sampling from one plant. High-throughput root sampling like "shovelomics" is possible in the field of ALRC. (D) Rinsed root system image captured using a 2D scanner. (E) Correlation between the total root lengths under hydroponic and sand dune field conditions.

et al. 2014) and soil compaction (Correa *et al.* 2019) may affect the RSA. The heterogeneity in soil water content and soil compaction in sand dune fields influence root growth and, by extension, explain the observed differences in the RSA between plants grown in sand dune fields and those raised hydroponically.

Root growth and growth period are limited under controlled conditions such as the use of vessels or pots for cultivation compared with those under field experiments. Rhizosphere size is also restricted under controlled conditions but clear root images may nonetheless be obtained by minimizing root loss. The root box-pin board method constrains the rhizosphere, but it is not suitable for sequential observations as the root box is several centimeters thick and the soil is completely rinsed off during root sampling. However, the whole root system may be obtained using this method because root loss and destruction are minimized (**Fig. 2**; Kono *et al.* 1987). The rhizotron is a nondestructive 2D root imaging method, but it also restricts the rhizosphere size. When soil is used for cultivation, the soil compounds interfere with image capture and prevent the acquisition of whole-root system images. The GLO-Roots system overcomes this problem using the *Arabidopsis thaliana*-transformed luminescence reporter gene (Rellán-Álvarez *et al.* 2015). Hydroponic, agar plate, and semi-hydroponic systems have been used for nondestructive root imaging and time-sensitive observations (Chen *et al.* 2020, Jeudy *et al.* 2016). Under these systems, the underground and sometimes the aboveground parts are relatively uniform. Hence, we can obtain highly reproducible data compared with those generated from soil and field experiments. However, the RSA under these conditions may differ from those for roots grown under controlled soil conditions (Ma *et al.* 2019). Hydrogel-based transparent soil may be useful for root phenotyping (Ma *et al.* 2019). This cost-effective system requires no specific nondestructive 3D imaging devices. The RSA of plants grown under hydrogel systems more closely resembles that of plants grown in soil than those for plants raised hydroponically. Thus, the hydrogel-bead system may mimic soil experiments (Ma *et al.* 2019).

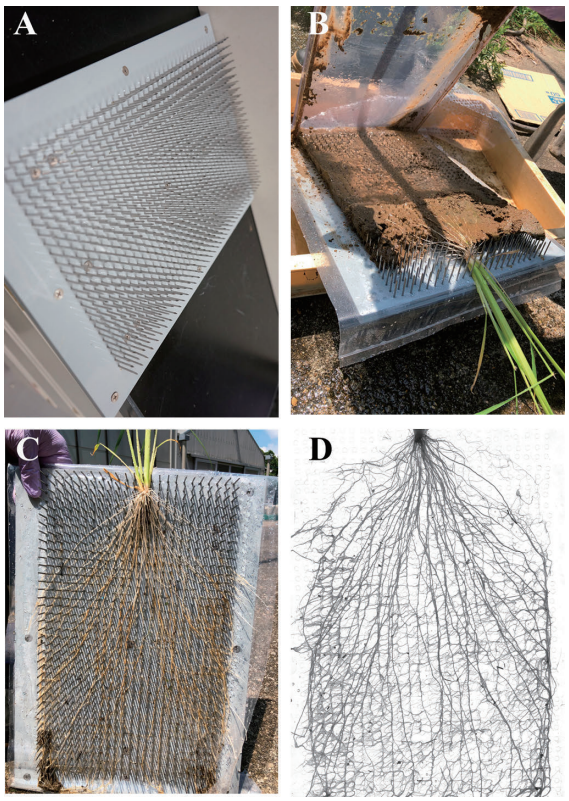

Fig. 2. Root box-pin board root sampling method. (A) Root sampling pin board. (B) Insertion of pin board plus plastic film into the soil in root box for collecting the whole root system. (C) Removal of root system from pin board with a plastic film after rinsing off the soil with water. (D) Root image prepared using a 2D scanner.

A current trend in root phenotyping is precise and non-destructive 3D imaging techniques such as magnetic resonance imaging (MRI) and X-ray computed tomography (X-ray CT). White neutron beam radiography and tomography are also used in root imaging (Shinohara *et al.* 2020, Tötzke *et al.* 2017). As water decays the neutron beam, these systems readily detect water and roots. Both plant RSA (Shinohara *et al.* 2020, Tötzke *et al.* 2017) and rhizosphere water content can be clearly visualized by neutron tomography (Tötzke *et al.* 2017). However, neutron beam irradiation may damage plant tissues. In contrast, MRI and X-ray CT have no such negative effects on plants (van Dusschoten *et al.* 2016, Zappala *et al.* 2013). Both MRI and X-ray CT may be combined with positron emission tomography (PET) to visualize carbon allocation (Garbout *et al.* 2012, Jahnke *et al.* 2009). While both techniques can produce clear 3D root images, X-ray CT can resolve them into thin root diameters (Metzner *et al.* 2015). Furthermore, X-ray CT is more widely used than MRI for root phenotyping as its cost is lower and non-medical X-ray CT scanners with vertical sample loading are available (Atkinson *et al.* 2019). Nevertheless, both techniques only slowly scan the RSA, reconstruct 3D images, and extract RSA traits. Teramoto *et al.* (2020) reported the development of a high-throughput rice (*Oryza sativa*) RSA phenotyping platform by X-ray CT. They optimized plant growth and CT scanning conditions and reduced the operating time. CT scanning and image processing could be completed in 10 min and 2–8 min, respectively, depending on hardware performance. However, restricted rhizosphere size is a potential limitation of these imaging methods. Soil water content and bias may affect root imaging by each method. Therefore, most studies use pots of diameter <100 mm in diameter. Nevertheless, the pot size may be enlarged to 200 mm by adjusting the soil type, soil particle size, scanning conditions, and/or root detection algorithms (Teramoto *et al.* 2020). These improvements could help facilitate non-destructive, large-scale RSA analyses, quantitative trait locus (QTL) assays, and genome-wide association studies (GWAS).

Phenotyping root structures and minimum information required for root modeling

The RSA is the result of interactions between genetically driven endogenous growth processes and environmentally determined exogenous constraints (Barthélémy and Caraglio 2007). Continuous root growth and ongoing branching create a highly complex network whose components have definite topology, geometry, and shape (Balduzzi *et al.* 2017). The root network structure or topology supports various fluxes such as the root hydraulic architecture. The interaction between the RSA and its environment depends mainly on 3D root distribution or the RSA geometry (Godin and Sinoquet 2005).

The complete RSA can be automatically or semi-automatically captured by root phenotyping image analysis software such as SmartRoot (Lobet *et al.* 2011) and RootNav (Pound *et al.* 2013). The Root System Markup Language format generates the RSA data on a standard XML format and is shared by various root phenotyping programs (Lobet *et al.* 2015). It involves root topology, geometry, and properties acquired from 2D or 3D images taken at one or more time points. Capturing the complete RSA of real soils is labor-intensive and has a low throughput. However, certain root traits can be evaluated at a relatively high throughput by manual measurements or using image analysis tools such as the Digital Imaging of Root Traits platform (Bucksch *et al.* 2014).

After extracting the root data, it is necessary to identify the traits that are meaningful and pertinent for a specific experiment. Hence, “root ideotype” for a particular research should be defined based on the specific environment that breeders are investigating. “Steep, cheap, and deep” has been proposed as the ideotype root system for maize (Lynch 2013). However, it may vary with soil environment. A deep root system (“deep”) is required because water percolates and nitrates leach into deeper soil strata (Lynch 2013). Under nitrate-deficient conditions, less lateral root branching and low crown root numbers (“steep”)

are suitable (Saengwilai *et al.* 2014, Zhan and Lynch 2015). However, as phosphorus, potassium, and ammonium are relatively immobile, short, shallow root systems with numerous lateral roots and long root hairs can more effectively utilize these resources (Lynch 2013). Therefore, root number, diameter, angle, and branching pattern are important traits in the ideotype for maize nitrogen and water acquisition. As it is important to identify the target (ideotype) root traits for each individual study, it might be useful to simplify an ideotype design by scoring the relative importance of root traits via root modeling. Certain root traits may be used to estimate the input parameters for functional-structural root models simulating RSA dynamics (Freschet *et al.* 2020, Pagès 2016). Functional-structural root models have been extensively used to investigate the relationships between root architectural traits and variability in soil and spatiotemporal nutrient distribution (Dunbabin *et al.* 2013, Ndour *et al.* 2017). These models simulate the structural and spatial distribution of the root system and directly integrate developmental processes such as elongation and branching, and their interaction with root-level soil properties (Pagès and Picon-Cochard 2014). Root architectural models using plant phenotyping data as input parameters have been recently developed. These include ArchiSimple (Pagès *et al.* 2012), DigR (Barczi *et al.* 2018), OpenSimRoot (Postma *et al.* 2017), and CRootBox (Schnepf *et al.* 2018). They were designed to represent interspecies root architectural diversity resulting from interactions with various environmental conditions (Muller *et al.* 2019, Pagès and Picon-Cochard 2014).

For each root category, most models define a set of parameters that specify the main processes such as emission, elongation, and ramification. Emission is specified by the maximum number of basal and shoot-bore roots and root growth time intervals. Elongation is defined by the elongation rate, root lifespan, and maximum root length. Branching is expressed by the length between ramifications, the ratio of the parent-to-daughter root diameter, and geometric data such as insertion angle and tropisms. These parameters must be measured for each root type. ArchiSimple was developed to reduce the number of required parameters (Pagès and Picon-Cochard 2014). It uses only the meristem size or apical diameter as a continuous descriptor of the root developmental capacity. It eliminates the requirement for multiple parameters for each root type and branching order. The meristem size is estimated by the tip diameter of young roots (see Freschet *et al.* 2020). In this model, the elongation rate varies with root diameter. A specific parameter defines the ratio of the lateral-to-mother root diameter and models the evolution of root length and elongation rate through branching orders. Lateral root growth pattern diversity is modeled by incorporating a stochastic function to assign lateral root diameter at emergence. The lateral root types emerge as a consequence of this stochasticity (Muller *et al.* 2019). The root primordium elongates only when its diameter exceeds a specified threshold. ArchiSimple has been used to characterize lateral root diversity in 140 monocot and dicot species (Pagès 2016). ArchiSimple requires only 13 parameters that can be estimated by excavating the RSA. The minimum diameter (D_{min}; smallest diameter of all elongated roots), maximum diameter (D_{max}; diameter of the primary root), and the relative diameter range (D_{range}) that characterizes diameter distribution. Diameter distribution is easily acquired using open-source image analysis software such as ImageJ with image data. The potential elongation rate is proportional to the diameter. EL is the slope of the root elongation rate versus the root tip diameter. EL can be approximated for some roots using their length, diameter at the tip (where the root is cylindrical), and their age. To capture branching, it is necessary to measure the interbranch distance on the thick roots (IBD) and the average ratio of diameter of the daughter root to that of the mother root (RDM). The IBD can be estimated from the branching density or directly from the image (**Fig. 3**). RDM is approximated by sampling some roots and mapping their tip diameter to the one of their mother roots. In **Fig. 3**, this can be achieved by computing the ratio of the tip diameter of the lateral roots to that of tap root. The final parameter is the coefficient of variation of the diameter of laterals (VarD). This factor allows the differentiation of homogeneous or highly variable laterals. The other parameters are the maximum number of adventitious roots (MNP), their emission rate (ER), the coefficient of growth duration (GD; links root growth duration to root diameter), the coefficient of gravitropism influencing root reorientation and representing plagiotropism or gravitropism, the coefficient of life duration for decay and abscission, and a parameter to compute radial growth (Pagès and Picon-Cochard 2014). These parameters would be easily obtained from "legacy root data", which have been preserved in old or unpublished formats. Scanning images may also be used for RSA imaging, but one of the simple legacy data may be photographs acquired using cameras in the field (**Fig. 3**). Here, we used field photographs as legacy data for simulations. The required root parameters were estimated manually. **Fig. 3** shows the reconstruction of two soybean genotypes using ArchiSimple. Model parameters were estimated from the legacy data by using image analysis software.

Conclusion and perspectives

Phenomics measures plant architectural traits in the canopy and root system. Phenotyping platforms facilitate these measurements of roots and shoots. Recent advances in root phenomics will enable large-scale, high-throughput, and precise RSA imaging. However, phenomics generally focuses only on the measurement and identification of phenotypic variation in plant traits rather than the relationship between phenotype and function. "Functional phenomics" attempts to describe the relationship between plant phenotype and physiological functions in the same way that

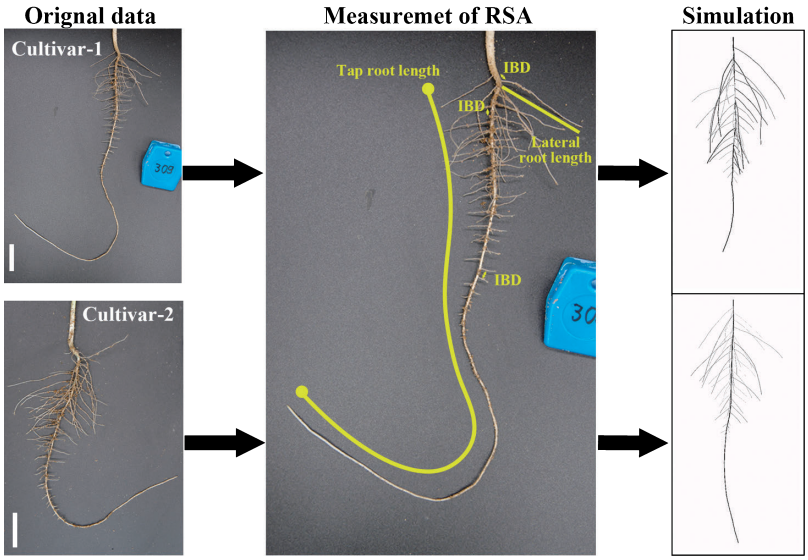

Fig. 3. Soybean root system reconstruction from simulation. The experimental field consists mainly of sand, and therefore, its water content was easy to control. Photographs prepared for RSA measurements in two different cultivars. The lateral root angle, lateral root number, tap and lateral root lengths, interbranch distance on lateral roots (IBD), and diameter range of lateral root including D_{max} and D_{min} were measured from photographs. Finally, root systems of the two genotypes at 7 days were simulated using ArchiSimple. Parameters of genotype 309 were as follows: D_{min} = 0.19 mm; D_{max} = 0.73 mm; EL = 34 day^{-1}; IBD = 1.48 mm; RMD = 0.41; VarD = 0.2 and angle = 76°. Parameters of genotype 324 were as follows: D_{min} = 0.09 mm; D_{max} = 0.70 mm; EL = 38 day^{-1}; IBD = 1.54 mm; RMD = 0.30; VarD = 0.28 and angle = 75°.

"functional genomics" shows the functions of genes and proteins and their interactions (York 2019). There are several important traits in functional root phenomics. Nodal root number and growth angle might correlate with shoot biomass and yield (Saengwilai *et al.* 2014, Slack *et al.* 2018 (https://doi.org/10.1101/280917), Wasson *et al.* 2012). Photosynthesis and photosynthate allocation influence total root length and alter root-shoot balance (York *et al.* 2013). Soil resource utilization and root ion uptake kinetics affect root growth and morphology (Griffiths and York 2020). The root cortical aerenchyma is an air space formed by programmed cell death. It reduces the number of living cortical cells, lowers the root segment respiration rates (Fan *et al.* 2003), and facilitates nutrient mobilization (Postma and Lynch 2011). The cortical area is determined by the aerenchyma, cortical cell size, and cortical cell file number (Jaramillo *et al.* 2013). The cortex-to-stele ratio contributes to crop tolerance to soil waterlogging (Yamauchi *et al.* 2019, reviewed in this issue by Yamauchi *et al.* 2021). Therefore, root anatomical features are important for understanding the relationships between root phenotype and plant growth. Ndour *et al.* (2017) presented a functional-structural drought stress RSA model including physiological, morphological, and anatomical data. Linking final yield or root phenotype to physiological, morphological, anatomical, and environmental data is a desirable direction for future RSA analysis. Rhizosphere microbiome composition may be associated with certain types of root morphology (Saleem *et al.* 2018) and domestication in the common bean (*Phaseolus vulgaris*) (Pérez-Jaramillo *et al.* 2017). Elucidation of the interactions between the rhizosphere microbiome and plant roots is important for breeding highly efficient roots.

To enhance root system efficiency, a whole-plant phenotyping platform associated with the RSA analysis should be developed under various environmental conditions (Tardieu *et al.* 2017). However, traditional root phenotyping methods and old, unpublished data should be still informative for the improvement of root system. Here, we identified and presented the minimum data required for root modeling. These minimum data could be easily extracted from the old format data. We hoped that the methodologies proposed in the present review can enable the re-use of these legacy data.

Author Contribution Statement

H.T. and C.P. wrote the manuscript. H.T. contributed to phenotyping. C.P. contributed to modeling.

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